Claims

What is claimed is:

1. A method for producing a polypeptide, comprising:

(a) cultivating a *Bacillus* cell in a medium conducive for the production of the polypeptide, wherein the *Bacillus* cell comprises a nucleic acid construct comprising a tandem promoter in which each promoter sequence of the tandem promoter is operably linked to a nucleic acid sequence encoding the polypeptide; and

(b) isolating the polypeptide from the cultivation medium.

2. The method of claim 1, wherein the nucleic acid construct further comprisies an mRNA processing/stabilizing sequence located downstream of the tandem promoter and upstream of the nucleic acid sequence encoding the polypeptide

3. The method of claim 1, wherein the tandem promoter comprises two or more bacterial promoter sequences.

4. The method of claim 3, wherein the two or more bacterial promoter sequences are obtained from one or more *Bacillus* genes.

5. The method of claim 1, wherein the tandem promoter comprises the *amyQ* promoter.

6. The method of claim 1, wherein the tandem promoter comprises a "consensus" promoter having the sequence TTGACA for the "-35" region and TATAAT for the "-10" region.

7. The method of claim 1, wherein the tandem promoter comprises the *amyL* promoter.

8. The method of claim 1, wherein the tandem promoter comprises the *cryIIIA* promoter.

9. The method of claim 1, wherein the tandem promoter comprises the *amyQ* promoter and the *cryIIIA* promoter.

10. The method of claim 1, wherein the tandem promoter comprises a "consensus" promoter having the sequence TTGACA for the "-35" region and TATAAT for the "-10" region and the *cryIIIA* promoter.

11. The method of claim 1, wherein the tandem promoter comprises the *amyL* promoter and the *cryIIIA* promoter.

12. The method of claim 1, wherein the tandem promoter comprises two copies of the *amyQ* promoter.

13. The method of claim 1, wherein the tandem promoter comprises two copies of a "consensus" promoter having the sequence TTGACA for the "-35" region and TATAAT for the "-10" region.

14. The method of claim 1, wherein the tandem promoter comprises two copies of the *amyL* promoter.

15. The method of claim 1, wherein the tandem promoter comprises two copies of the *cryIIIA* promoter.

16. The method of claim 1, wherein the two or more promoter sequences of the tandem promoter simultaneously promote the transcription of the nucleic acid sequence.

17. The method of claim 1, wherein one or more of the two or more promoter sequences of the tandem promoter promote the transcription of the nucleic acid sequence at different stages of growth of the *Bacillus* cell.

18. The method of claim 1, wherein the mRNA processing/stabilizing sequence is the *cryIIIA* mRNA processing/stabilizing sequence.

19. The method of claim 1, wherein the mRNA processing/stabilizing sequence is the SP82 mRNA processing/stabilizing sequence.

20. The method of claim 1, wherein the mRNA processing/stabilizing sequence generates mRNA transcripts essentially of the same size.

21. The method of claim 1, wherein the *Bacillus* cell contains one or more copies of the nucleic acid construct.

22. The method of claim 1, wherein the *Bacillus* cell contains one copy of the nucleic acid construct.

23. The method of claim 1, wherein the nucleic acid construct further comprises a selectable marker gene.

24. The method of claim 1, wherein the *Bacillus* cell contains no selectable marker gene.

25. The method of claim 1, wherein the nucleic acid sequence encodes a polypeptide heterologous to the *Bacillus* cell.

26. The method of claim 1, wherein the polypeptide is a hormone or variant thereof, enzyme, receptor or portion thereof, antibody or portion thereof, or reporter.

27. The method of claim 26, wherein the enzyme is an oxidoreductase, transferase, hydrolase, lyase, isomerase, or ligase.

28. The method of claim 27, wherein the enzyme is an aminopeptidase, amylase, carbohydrase, carboxypeptidase, catalase, cellulase, chitinase, cutinase, cyclodextrin glycosyltransferase, deoxyribonuclease, esterase, alpha-galactosidase, beta-galactosidase, glucoamylase, alpha-glucosidase, beta-glucosidase, invertase, laccase, lipase, mannosidase, mutanase, oxidase, a pectinolytic enzyme, peroxidase, phytase, polyphenoloxidase, proteolytic enzyme, ribonuclease, transglutaminase, or xylanase.

29. The method of claim 1, wherein the nucleic acid sequence is contained in the chromosome of the *Bacillus* cell.

30. The method of claim 1, wherein the nucleic acid sequence is contained on an extrachromosomal element.

31. The method of claim 1, wherein the *Bacillus* host cell is a *Bacillus alkalophilus*, *Bacillus amyloliquefaciens*, *Bacillus brevis*, *Bacillus circulans*, *Bacillus clausii*, *Bacillus coagulans*, *Bacillus firmus*, *Bacillus lautus*, *Bacillus lentus*, *Bacillus licheniformis*, *Bacillus megaterium*, *Bacillus pumilus*, *Bacillus stearothermophilus*, *Bacillus subtilis*, or *Bacillus thuringiensis* cell.

32. The method of claim 31, wherein the *Bacillus* cell is a *Bacillus subtilis* cell.

33. A *Bacillus* cell comprising a nucleic acid construct which comprises (a) a tandem promoter in which each promoter sequence of the tandem promoter is operably linked to a single copy of a nucleic acid sequence encoding a polypeptide, and optionally (b) an mRNA processing/stabilizing sequence located downstream of the tandem promoter and upstream of the nucleic acid sequence encoding the polypeptide.

34. The cell of claim 33, wherein the nucleic acid construct further comprises a selectable marker gene.

35. The cell of claim 33, which contains no selectable marker gene.

36. A method for obtaining a *Bacillus* host cell, comprising introducing into a *Bacillus* cell a nucleic acid construct comprising (i) a tandem promoter in which each promoter sequence of the tandem promoter is operably linked to a single copy of a nucleic acid sequence encoding a polypeptide and alternatively also (ii) an mRNA processing/stabilizing sequence located downstream of the tandem promoter and upstream of the nucleic acid sequence encoding the polypeptide.

37. A method for producing a selectable marker-free mutant of a *Bacillus* cell, comprising deleting a selectable marker gene of the *Bacillus* cell, wherein the *Bacillus* cell comprises a nucleic acid construct comprising (i) a tandem promoter in which each promoter sequence of

the tandem promoter is operably linked to a single copy of a nucleic acid sequence encoding a polypeptide and alternatively also (ii) an mRNA processing/stabilizing sequence located downstream of the tandem promoter and upstream of the nucleic acid sequence encoding the polypeptide.

38. A selectable marker-free mutant of a *Bacillus* cell obtained by the method of claim 37.

39. A method for producing a polypeptide, comprising:

(a) cultivating a *Bacillus* cell in a medium conducive for the production of the polypeptide, wherein the *Bacillus* cell comprises a nucleic acid construct comprising (i) a "consensus" promoter having the sequence TTGACA for the "-35" region and TATAAT for the "-10" region operably linked to a single copy of a nucleic acid sequence encoding the polypeptide and (ii) an mRNA processing/stabilizing sequence located downstream of the "consensus" promoter and upstream of the nucleic acid sequence encoding the polypeptide; and

(b) isolating the polypeptide from the cultivation medium.

40. The method of claim 39, wherein the consensus promoter is obtained from any bacterial promoter.

41. The method of claim 40, wherein the "consensus" promoter is obtained from a *Bacillus* promoter.

42. The method of claim 40, wherein the consensus promoter is obtained from a promoter obtained from the *E. coli lac* operon *Streptomyces coelicolor* agarase gene (*dagA*), *Bacillus lentus* alkaline protease gene (*aprH*), *Bacillus licheniformis* alkaline protease gene (subtilisin Carlsberg gene), *Bacillus subtilis* levansucrase gene (*sacB*), *Bacillus* subtilis alpha-amylase gene (*amyE*), *Bacillus licheniformis* alpha-amylase gene (*amyL*), *Bacillus stearothermophilus* maltogenic amylase gene (*amyM*), *Bacillus amyloliquefaciens* alpha-amylase gene (*amyQ*), *Bacillus licheniformis* penicillinase gene (*penP*). *Bacillus subtilis xylA* and *xylB* genes, *Bacillus thuringiensis* subsp. *tenebrionis* CryIIIA gene (*cryIIIA*, SEQ ID NO. 21) or portions thereof, or prokaryotic beta-lactamase gene *spo1* bacterial phage promoter.

43. The method of claim 40, wherein the "consensus" promoter is obtained from the *Bacillus amyloliquefaciens* alpha-amylase gene (*amyQ*).

44. The method of claim 43, wherein the "consensus" *amyQ* promoter has the nucleic acid sequence of SEQ ID NO. 26 or SEQ ID NO. 27.

45. The method of claim 39, wherein the mRNA processing/stabilizing sequence is the *cryIIIA* mRNA processing/stabilizing sequence.

46. The method of claim 39, wherein the mRNA processing/stabilizing sequence is the SP82 mRNA processing/stabilizing sequence.

47. The method of claim 39, wherein the mRNA processing/stabilizing sequence generates mRNA transcripts essentially of the same size.

48. The method of claim 39, wherein the *Bacillus* cell contains one or more copies of the nucleic acid construct.

49. The method of claim 39, wherein the *Bacillus* cell contains one copy of the nucleic acid construct.

50. The method of claim 39, wherein the nucleic acid construct further comprises a selectable marker gene.

51. The method of claim 39, wherein the *Bacillus* cell contains no selectable marker gene.

52. The method of claim 39, wherein the nucleic acid sequence encodes a polypeptide heterologous to the *Bacillus* cell.

53. The method of claim 39, wherein the polypeptide is a hormone or variant thereof, enzyme, receptor or portion thereof, antibody or portion thereof, or reporter.

54. The method of claim 53, wherein the enzyme is an oxidoreductase, transferase, hydrolase, lyase, isomerase, or ligase.

55. The method of claim 53, wherein the enzyme is an aminopeptidase, amylase, carbohydrase, carboxypeptidase, catalase, cellulase, chitinase, cutinase, cyclodextrin glycosyltransferase, deoxyribonuclease, esterase, alpha-galactosidase, beta-galactosidase, glucoamylase, alpha-glucosidase, beta-glucosidase, invertase, laccase, lipase, mannosidase, mutanase, oxidase, a pectinolytic enzyme, peroxidase, phytase, polyphenoloxidase, proteolytic enzyme, ribonuclease, transglutaminase, or xylanase.

56. The method of claim 39, wherein the nucleic acid sequence is contained in the chromosome of the *Bacillus* cell.

57. The method of claim 39, wherein the nucleic acid sequence is contained on an extrachromosomal element.

58. The method of claim 39, wherein the *Bacillus* host cell is a *Bacillus alkalophilus*, *Bacillus amyloliquefaciens*, *Bacillus brevis*, *Bacillus circulans*, *Bacillus clausii*, *Bacillus coagulans*, *Bacillus firmus*, *Bacillus lautus*, *Bacillus lentus*, *Bacillus licheniformis*, *Bacillus megaterium*, *Bacillus pumilus*, *Bacillus stearothermophilus*, *Bacillus subtilis*, or *Bacillus thuringiensis* cell.

59. The method of claim 39, wherein the *Bacillus* cell is a *Bacillus subtilis* cell.

60. A *Bacillus* cell comprising a nucleic acid construct which comprises (a) a "consensus" promoter having the sequence TTGACA for the "-35" region and TATAAT for the "-10" region operably linked to a single copy of a nucleic acid sequence encoding the polypeptide and (b) an mRNA processing/stabilizing sequence located downstream of the "consensus" promoter and upstream of the nucleic acid sequence encoding the polypeptide.

61. The cell of claim 60, wherein the nucleic acid construct further comprises a selectable marker gene.

62. The cell of claim 60, which contains no selectable marker gene.

63. A method for obtaining a *Bacillus* host cell, comprising introducing into a *Bacillus* cell a nucleic acid construct comprising (i) a "consensus" promoter having the sequence TTGACA for the "-35" region and TATAAT for the "-10" region operably linked to a single copy of a nucleic acid sequence encoding the polypeptide and (ii) an mRNA processing/stabilizing sequence located downstream of the "consensus" promoter and upstream of the nucleic acid sequence encoding the polypeptide.

64. A method for producing a selectable marker-free mutant of a *Bacillus* cell, comprising deleting a selectable marker gene of the *Bacillus* cell, wherein the *Bacillus* cell comprises a nucleic acid construct comprising (i) a "consensus" promoter having the sequence TTGACA for the "-35" region and TATAAT for the "-10" region operably linked to a single copy of a nucleic acid sequence encoding the polypeptide and (ii) an mRNA processing/stabilizing sequence located downstream of the "consensus" promoter and upstream of the nucleic acid sequence encoding the polypeptide.

65. A selectable marker-free mutant of a *Bacillus* cell obtained by the method of claim 64.

66. An isolated "consensus" *amyQ* promoter sequence having the nucleic acid sequence contained in SEQ ID NO. 26 or SEQ ID NO. 27.

67. A nucleic acid construct comprising a nucleic acid sequence encoding a polypeptide operably linked to one or more copies of the promoter of claim 66.

68. A recombinant expression vector comprising the nucleic acid construct of claim 67.

69. A recombinant *Bacillus* cell comprising the nucleic acid construct of claim 67.

70. A method for producing a polypeptide, comprising: (a) cultivating a *Bacillus* cell in a medium conducive for the production of the polypeptide, wherein the *Bacillus* cell comprises a nucleic acid sequence encoding the polypeptide operably linked to one or more copies of the

"consensus" *amyQ* promoter of claim 65; and (b) isolating the polypeptide from the cultivation medium.

71. A method for producing a selectable marker-free mutant of a *Bacillus* cell, comprising deleting a selectable marker gene of the *Bacillus* cell, wherein the *Bacillus* cell comprises a nucleic acid sequence encoding the polypeptide operably linked to one or more copies of the "consensus" *amyQ* promoter of claim 66.

72. A selectable marker-free mutant of a *Bacillus* cell obtained by the method of claim 71.

73. The method of claim 1, wherein the *Bacillus* cell comprises only one copy of the nucleic acid sequence.